(12) UK Patent Application (19) GB (11) 2 342 992 (13) A

(43) Date f A Publication 26.04.2000

(21) Application No 9823177.2

(22) Date of Filing 22.10.1998

(51) INT CL[7]
G01N 33/543

(52) UK CL (Edition R)
G1B BAE B307 B309 B402 B423

(56) Documents Cited
WO 91/10747 A1

(58) Field of Search
UK CL (Edition Q) G1B BAE
INT CL[6] G01N 33/53 33/58

(71) Applicant(s)
Horticulture Research International
(Incorporated in the United Kingdom)
Wellesbourne, WARWICK, CV35 9EF, United Kingdom

(72) Inventor(s)
Michael Frederick Clark
Nigel Frederick Lyons

(74) Agent and/or Address for Service
Marks & Clerk
57-60 Lincoln's Inn Fields, LONDON, WC2A 3LS,
United Kingdom

(54) Abstract Title
Dipstick detection system

(57) A two-step capillary flow immunoassay comprises the steps of:
- contacting a wicking strip with a sample containing an analyte-specific, biotinylated antibody such that said antibodies may complex with an immobilised immunoreactant, wherein said immunoreactant is either an antibody specific to the analyte or the analyte itself, wherein the analyte is preferably an antigen or a hapten and wherein said wicking strip may also include an immobilised control antibody,
- contacting said wicking strip with a gold-labelled, biotin-specific antibody.

Also claimed is a two-step capillary flow immunoassay kit for carrying out the above procedure.

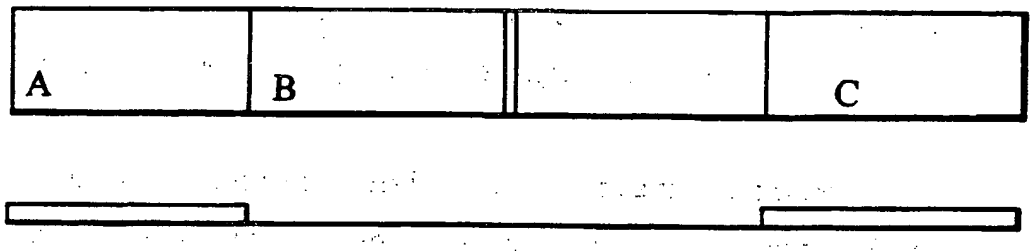
A
B
C

Fig. 1

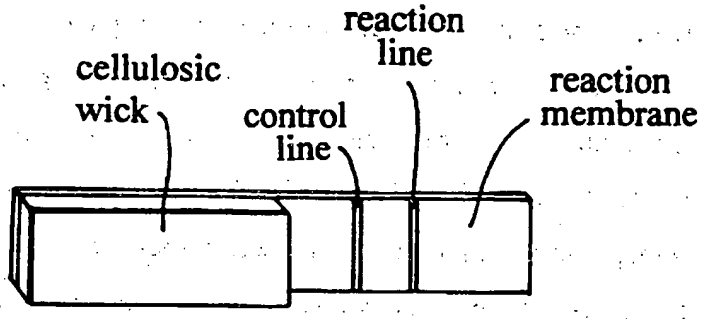
cellulosic wick
control line
reaction line
reaction membrane

Fig. 2

DIP-STICK DETECTION SYSTEM

The present invention relates to a detection system.

Lateral flow or dipstick technology was originally developed as a simple home-use pregnancy test. It has subsequently been adapted for many other uses in veterinary and clinical medicine but the principles of the test system have remained unchanged.

The basic design is a combination of three components (shown in Fig 1). A is the sample application area, B is the nitro-cellulose membrane to which a thin stripe of specific antibody (usually monoclonal) has been applied and is immobilised, C is the absorbent wick.

In the majority of commercially available devices the same specific antibody (as in B) but coupled to colloidal gold particles (occasionally coloured latex beads), is pre-loaded onto the sample area and the liquefied sample is applied directly to A. If the target component (antigen) is present in the sample the gold-labelled antibody attaches to it and is drawn with the liquid flow through the nitro-cellulose membrane. When the antigen reaches the stripe of immobilised antibody it is captured and prevented from continuing with the liquid flow into the absorbent wick. As the antigen has been coated with the gold-labelled antibody this is concentrated on the stripe and a coloured line develops. Where no target antigen is present in the sample all the gold-labelled antibody is drawn into the absorbent wick and no line develops.

In practice most devices also have an additional component immobilised as a second stripe on the membrane which interacts either directly with colloidal gold, or through excess gold-labelled antibody. This constitutes a control to indicate the correct functioning of the test. In this case the development of two coloured lines indicates a positive sample. If only the control line becomes visible the test is deemed negative but proof that the test device is working correctly.

For most tests utilising this technology the components are housed in a cassette made of plastic or other impervious material.

The present invention is concerned with a universal test system for use with polyclonal or monoclonal antibodies, or with a mixture of both. The same basic design is used for direct tests in which the presence of the target analyte (antigen) is signalled by the development of a visible, coloured reaction line, and for competition tests in which the presence of the target analyte (hapten) prevents the development of the reaction line. The system is highly sensitive and allows very rapid results (<20 min).

SUMMARY OF INVENTION

The present invention provides a two-step capillary flow immunoassay where firstly sample with biotinylated antibody specific to the analyte is applied to a wicking strip to flow to encounter an immobilised immunoreactant which is either antibody specific to the analyte or is the analyte, and optionally to flow to an immobilised control antibody, and secondly gold-labelled antibody specific to biotin is applied.

The present invention provides the assay procedure, and also provides component parts for the assay. In particular, the present invention provides a two-step capillary flow immunoassay kit comprising a wicking strip with an immobilised immunoreactant which is either antibody specific to the analyte or is the analyte, and optionally an immobilised control antibody, a supply of biotinylated antibody specific

the analyte, and gold-labelled antibody specific to biotin.

PREFERRED EMBODIMENTS

There are currently two particularly preferred embodiments, direct antigen detection and competitive hapten detection.

Components of the system for direct antigen detection are shown in Fig 2.

The device is in the form of a stick comprising a free-standing (non-enclosed) membrane (commercially available) connected via an adhesive backing strip to a cellulosic wick. The stick has been designated, provisionally, an "EMC-Stick". Purified antibody specific for the antigen to be detected (capture antibody) is immobilised in a stripe on the reaction membrane at a position referred to as the reaction line.

An anti-species antibody is similarly immobilised at the control line position.

Purified antibody specific for the antigen to be detected is conjugated with the small water-soluble vitamin biotin, using a commercially available biotinylating reagent (detecting antibody). ~~Any suitable biotinylation system may be used in the present invention.~~ The ratio of biotin molecules to antibody is suitably controlled. ~~The present invention also suitably employs~~ Colloidal gold conjugated with antibody specifically reactive with biotin (commercially available) ~~see Example 1).~~

Aqueous buffer solutions are optimised for detection of the target antigen.

The present antigen detection system is run in two stages. In the first stage an aqueous suspension of the material under test (made using an optimised buffer mixture) is treated with the biotinylated detecting antibody conjugate. If target antigen is present in the aqueous suspension, biotin-labelled antibody will bind to it. The stick is positioned in the container with a measured amount of the mixture of aqueous test material and biotinylated antibody, so that the tip of the reaction membrane is immersed in the mixture of a distance of 2-3 mm. The aqueous mixture wicks up the membrane by capillary action, passing through the reaction line and control line positions. Any antigen in the mixture is captured by an immobilised antibody at the site of the reaction line and both the antigen and its attendant biotin label accumulates. Excess (unreacting) antibody passes through the reaction site and some is captured by the anti-species antibody immobilised at the control line. Liquid continues to wick through the membrane into the cellulosic filter until either all the liquid has been drained out of the container or the EMC-stick is physically removed.

In the second stage, the EMC-stick is repositioned in a second container in which has been placed a measured amount of a suspension of size-optimised (usually 40 nm) colloidal gold labelled with antibody specifically reactive with biotin (commercially available). The anti-biotin gold conjugate wicks up the membrane by capillary action. Where the conjugate encounters biotin - at the reaction line if target antigen is present and the control line, the antibody binds to the biotin with concomitant accumulation of colloidal gold. The is results in the formation of highly visible red lines. Hence a positive reaction is one in which two visible reaction zones appear, at the reaction line and the control line positions. A negative reaction is one in which a red line appears only at the control line position. Excess (unreacting) gold conjugate is drawn through the membrane into the wick.

Components of the system for competitive hapten (e.g. pesticide) detection are shown in Fig 2.

The device is in the form of a stick comprising a free-standing (non-enclosed) membrane (commercially available) connected via an adhesive backing strip to a cellulosic wick. The stick has been designated, provisionally, and "EMC-Stick". A preparation of hapten molecules, conjugated to an inert carrier protein (e.g. bovine serum albumin) is immobilised via the carrier protein in a stripe on the reaction membrane at a position referred to as the reaction line.

An anti-species antibody is similarly immobilised at the control line position.

Purified antibody specific for the hapten to be detected is conjugated with the small water-soluble vitamin biotin, using a commercially available biotinylating reagent (detecting antibody). The ratio of biotin molecules to antibody is suitably controlled. Colloidal gold conjugated with antibody specifically reactive with biotin (commercially available).

Aqueous buffer solution is optimised for detection of the target hapten.

The hapten detection system is run in two stages. In the first stage an aqueous suspension of the material under test (made using an optimised buffer mixture) is treated with the limiting concentration of the biotinylated detecting antibody conjugate. If target hapten is present in the aqueous suspension, biotin-labelled antibody will bind to it. The EMC-stick is positioned in the container with a measured amount of the mixture of aqueous test material and biotinylated antibody, so that the tip of the reaction membrane is immersed in the mixture for a distance of 2-3 mm. The aqueous mixture wicks up the membrane by capillary action, passing through the reaction line and control line positions. Any hapten in the mixture will have already bound to the

capture antibody, thus preventing the biotinylated antibody from reacting with the immobilised hapten-protein conjugate on the membrane. If none, or very little hapten is present in the test material, competition for the biotinylated capture antibody does not occur and the antibody is free to bind to the immobilised hapten-protein conjugate. Excess (unreacting) antibody passes through the reaction site and some is captured by the anti-species antibody immobilised at the control line. Liquid continues to wick through the membrane into the cellulosic filter until either all the liquid has been drained out of the container or the EMC-stick is physically removed.

In the second stage, the EMC-stick is repositioned in a second container in which has been placed a measured amount of a suspension of size-optimised (usually 40 nm) colloidal gold labelled with antibody specifically reactive with biotin (commercially available). The anti-biotin gold conjugate wicks up the membrane by capillary action. Where the conjugate encounters biotin - at the reaction line if target hapten is absent and the control line, - the antibody binds to the biotin with concomitant accumulation of colloidal gold. This results in the formation of highly visible red lines. Hence a positive reaction is one in which only one visible reaction zone appears, at the control line position. A negative reaction is one in which hapten competition for the biotinylated antibody does not occur and thus two red lines develop, at the reaction line as well as the control line positions. Excess (unreacting) gold conjugate is drawn through the membrane into the wick.

The application of this type of dipstick format is thought to be unique for the detection of plant-derived antigens and haptens.

The use of biotin to label polyclonal and/or monoclonal antibodies specific for plant-derived antigens and haptens, in conjunction with anti-biotin colloidal gold, is unique to the recognition system of this EMC-stick system.

This technique has been developed specifically for use both by non-technical personnel as well as by laboratory-trained personnel.

The sensitivity of the method for bacteria has been evaluated in comparison with an enzyme-linked immunoassay, ELISA, giving about the same sensitivity. For pesticide detection such as metaxyl, a comparison with gas chromatography/mass spectrometry showed equal sensitivity, which was also confirmed using ELISA. The detection was around 100 pg/mg, or 0.1 parts per million.

CLAIMS

1. A two-step capillary flow immunoassay where firstly sample with biotinylated antibody specific to the analyte is applied to a wicking strip to flow to encounter an immobilised immunoreactant which is either antibody specific to the analyte or is the analyte, and optionally to flow to an immobilised control antibody, and secondly gold-labelled antibody specific to biotin is applied.

2. The immunoassay of claim 1, when in the form of a direct antigen immunoassay.

3. The immunoassay of claim 1, when in the form of a competitive hapten immunoassay.

4. A two-step capillary flow immunoassay kit comprising a wicking strip with an immobilised immunoreactant which is either antibody specific to the analyte or is the analyte, and optionally an immobilised control antibody, a supply of biotinylated antibody specific to the analyte, and gold-labelled antibody specific to biotin.

The Patent Office

INVESTOR IN PEOPLE

Application No:	GB 9823177.2	**Examiner:**	Cass Dottridge
Claims searched:	1-4	**Date f search:**	9 April 1999

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.Q): G1B BAE

Int Cl (Ed.6): G01N 33/53-33/58

Other: ONLINE: WPI, EDOC, CAS-ONLINE.

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X	WO 91/10747 A1 (PRUTECH RES. DEV. PARTNERSHIP) page 10, lines 4-31	1-4

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.